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RACIAL VARIATION OF INTERLEUKIN-6 IN SOCCER PLAYERS: THE EFFECT OF SHORT-TERM MAXIMAL EXERCISE

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ABSTRACT

Inflammation is a common pathophysiological pathway for a number of chronic diseases. This study examined the effects of time-of-day and race on soccer players' plasma concentration of interleukin-6 (IL-6) during short-term maximal exercise. 36 soccer players were divided in three groups (i.e., 12 black Tunisian (BT), 12 white Tunisian (WT), and 12 south African (SA)) and performed a 30-s Wingate test at 08:00 and 18:00 h during which the peak (P_{peak}) and mean (P_{mean}) powers and the fatigue index (FI) were assessed. Moreover, plasma interleukin-6 (IL-6) concentrations were measured before (P1) and immediately (P2) and 60 min (P3) after the exercise. Our results showed that oral temperature, P_{peak} and P_{mean} were significantly higher ($p < 0.001$) in the afternoon than in the morning and considerably more so in SA than in WT and BT ($p < 0.05$). However, no major difference was observed between WT and BT in regards to all parameters. Likewise, plasma concentration of IL-6 were significantly higher ($P<0.01$) in the afternoon than in the morning in all groups. Furthermore, IL-6 levels were significantly higher in SA than WT and BT subjects ($p<0.05$) regardless of the time of test. In addition, IL-6 levels were significantly higher in all groups at P2 than P1 and P3 ($p<0.001$). However, no significant difference in IL-6 level was observed between WT and BT, and between P1 and P3. The higher concentration of IL-6 may reflect the susceptibility of South African to inflammatory diseases, which could be important in assessing health disparities among Blacks and Whites athletes. Furthermore, results from the field of exercise immunology may help to guide athletes and contribute to public health recommendations on exercise and infections.

KEYWORDS: Ethnic, Anaerobic Performance, IL-6, Circadian Rhythm, Diurnal

INTRODUCTION

It is clear that black athletes dominate anaerobic performance sports **(Boulay et al., 1988; Malina, 1986)**. In this context, we recently found that black Tunisian males presented higher aerobic and anaerobic performances than white Tunisian males **(Abedelmalek et al., 2012a)**. Moreover, **Ben Ayed et al. (2011)** showed that black Tunisian footballers presented higher jumping and sprinting performance than white Tunisian. Therefore, exercise physiologists attempted to explain this supremacy for the blacks by (i) a higher anaerobic capacity compared to whites **(Ama et al, 1986)** (ii) the largest percentage of fast-twitch muscle fibres **(Ama et al, 1990)**, and (iii) an increased glycolytic enzyme activity responsible for the production of energy from anaerobic metabolism of carbohydrate. Likewise, environmental factors, socioeconomic status, and cycling skills have been found as underlying racial or ethnic variation **(Himes, 1988; Malina, 1988; De Jonge et al, 1996)**

Nevertheless, in these studies the time-of-day effect on anaerobic performances has not been taken into consideration, even though the effects of the circadian rhythm on short-term physical performance have been well established **(Chtourou et al., 2012)**. In this context, short-term maximal performance is typically better at the afternoon, around the peak of the circadian rhythm of body temperature **(Abedelmalek et al., 2012b; Chtourou et al., 2012a, b, c)**.

On the other hand, regarding the effects of the circadian rhythm on immunological variables, data from the literature suggested that an interaction among circadian rhythms and pro-inflamatory cytokine such as Interleukin-6 (IL-6) **(Redwine et al., 2000; Vgontzas et al., 2004)**. Indeed, circulating concentrations of IL-6 show a diurnal fluctuation, with lower values during the daytime and maximum value at night. The IL-6 is a pro-inflammatory cytokine that also has an important role in immunity. Moreover, IL-6 is a pleiotropic cytokine and its secretion is stimulated by physiological, psychological and pathological stressors. It has been shown that high-intensity exercise induces immune-suppression and may explain the increased risk of infection in athletes **(Meckel et al., 2009)**. Recently, **Meckel et al., (2011)** showed that a repeated-sprint exercise was associated with a significant increase in the plasma IL-6 that may indicate its important role in muscle tissue repair post-exercise in trained subjects. Likewise, more recently, we showed a significant increase of IL-6 after the Wingate test in sleep deprived soccer players **(Abedelmalek et al., 2012b)**. Less is known about factors that may influence the inflammatory response in athletes of distinct ethnic backgrounds. **Chapman et al., (2009)** showed considerable individual genders and/or racial variation in inflammatory parameters. Likewise, **Paalani et al., (2011)** showed that higher IL-6 levels were observed in black as compared to white subjects. Similar findings have been observed by **Cruciani et al., (2008) and Ciarleglio et al., (2008)**. Moreover, **Cohen et al., (2006)** showed that black subjects had higher levels of cortisol during the evening hours in both men and women.

Although there are some evidences of the difference in daily Il-6 levels between race/ethnicity, the current literature present inconclusive findings. Moreover, to the authors' knowledge, no previous study has investigated the impact of race/ethnicity on the diurnal fluctuation of IL-6 during a short-term anaerobic exercise. Therefore, in the present study we examined the effects of time-of-day and race on soccer players' plasma concentration of interleukin-6 (IL-6) during short-term maximal exercise

METHODS

Subjects

Thirty six healthy soccer players, 12 black Tunisian (BT), 12 white Tunisian (WT) from the same Berber descent **(Hajjej et al., 2006)**, and 12 black South African (SA) (from Cameroon, Senegal, Zaire, Ivory Coast, and Burundi) participated in the study after having a thorough explanation of the protocol and signing a consent document. Subjects' characteristics were presented in Table 1. Based on the chronotype questionnaire the subjects had an "intermediate chronotype". Subjects reported no sleep disorder, no smoking habit, no consumption of caffeine or any alcoholic beverages and none of them was taking any medication. The study protocol complied with the Helsinki declaration for human experimentation and was approved by the University Ethics Committee.

Table 1: Characteristics of Subjects

	Black Tunisian (n=12)	White Tunisian (n=12)	South African (n=12)	P Values
Age (years)	21.25±0.54	22.06±2.171	21.20±1.28	0.63
Height (cm)	177.2±0.06	178±0.05	174.3±0.02	0.44
Weight (Kg)	72.48±6.36	75.95±2.204	73.5±1.74	0.44
BMI (Kg /m^2)	24.15±2.21	23.87±1.54	24.21±0.81	0.46

BMI: Body Mass Index

Protocol

Before the start of the experimental period, the subjects were familiarized with the cycle-ergometer and high-velocity cycling exercises so as to minimize potential learning effects during the period of the investigation (**Abedelmalek et al., 2012b)**. Then, they performed two Wingate tests, one in the morning at 08:00 h and one in the evening at 18:00 h in a randomized order over two days with a recovery period of at least 34-h in-between. These time points were chosen as they are generally reported in the literature as phases of the minimum and maximum daytime levels of power output during the Wingate test. Upon arrival to the laboratory and after resting for 15 min, oral temperature was measured during each test session by a digital clinical thermometer (Omron, Paris, France; accuracy ±0.05°C) inserted sublingually for at least 3 min. The experimental trials were conducted as follows: 30 min of rest including participant's preparation (e.g., measurement of oral temperature and body weight and height), 5 min of active warm-up (i.e., pedalling at 60 rpm with 2-3-s all-out sprints after each 1 min with 1kg) on a cycle ergometer, a recovery period of 5 min, and subsequently a 30-s Wingate test. Body weight was measured to the nearest of 0.1 kg using a Tanita digital scale (Tanita, Tokyo, Japan).

On the night preceding each test session, subjects were asked to keep their usual sleeping habits, with a minimum of 6-h sleep. Moreover, participants were requested to maintain their habitual physical activity and to avoid strenuous activity in the 24-h before each test session **(Chtourou et al., 2012d)**. On the day of the morning test session, subjects were instructed to wake up at 07:00 h (1-h before the test session) and only one glass of water (150 to 200 ml) was allowed. On the day of the evening test session, they had the same standard isocaloric meal at 12:00 h. After that meal, only water was allowed ad libitum.

OUTCOME MEASURES

Blood Samples and Analyses

Blood samples were taken using an indwelling venous catheter before (i.e., after 10-15 min of rest), immediately after and 60 min after the exercise and were centrifuged at 3000 rpm for 10 min at room temperature. Plasma samples were stored at -80 °C until the measurement of immune parameters. Prior to statistical analyses, all data were corrected for changes in plasma volume using the method of Costill and Fink (1974). All the assays were carried out as advised by manufacturer's directions. To eliminate inter-assay variance, all samples for each subject were assayed in the same assay.

Plasma concentration of IL-6 was analyzed by ELISA (i.e., Enzyme Linked Immuno-Assay) with the use of the commercial kit (Immunotech, Marseille, France). The intra-assay and the inter-assay coefficients of variation were 1.6-6.8% and 7.9-14.6% respectively.

Exercise Protocol

The Wingate test involved a 30-s maximal sprint against constant resistance equal to 0.087 $kg \cdot kg^{-2}$ body mass of the subject **(Chtourou et al., 2012c)**. Seat height was adjusted to each participant's satisfaction and kept the same for each participant throughout the two trials. Participants were given vigorous verbal encouragement during the test. Toe-clips were used to prevent the participant's feet from slipping off the pedals.

P_{peak}, P_{mean}, and the fatigue index (FI) were recorded at the end of the test. As previously determined, the FI was calculated as follow:

$$FI\ (\%) = ((P_{peak} - P_{lowest})/P_{peak}) \times 100$$

With P_{lowest} is the lowest power observed during the 30-s of the exercise.

Statistical Analysis

Statistical tests were processed using STATISTICA Software (Stat Soft, France). Data are reported as mean ± SD. The assumption of normality was confirmed using the Shapiro-Wilk *W*-test, parametric tests were performed. The oral temperature and anaerobic performance (i.e., P_{peak}, P_{mean}, and FI) were determined using a two-way analysis of variance (ANOVA) (2 [time-of-day] × 3 [groups]). Inflammatory mediators were determined using a 3-way ANOVA (2 [time-of-day] × 3 [groups] × 3 [points of measurement]). When appropriate, significant differences among means were tested using the LSD post-hoc test. The level of statistical significance was set at $p < 0.05$.

RESULTS

Plasma Concentrations of Interleukin-6

There were significant main effects of groups ($F_{(2,14)} = 14.51$; $P<0.001$), time-of-day ($F_{(1,7)} = 80.36$; $P<0.001$) and points of measurement ($F_{(2,14)} = 46.68$; $P<0.05$). Likewise, there were significant interaction effects of time-of-day × groups ($F_{(2,14)} = 6.58$; $P<0.05$) and time-of-day × points of measurement ($F_{(2.14)} = 47.22$; $P<0.001$) (Table 2). However, the interaction of time-of-day × groups × points of measurement was not significant

Post Hoc results revealed that IL-6 levels were significantly higher in the afternoon than the morning in WT ($p<0.05$), BT, ($p<0.05$) and SA ($p<0.001$). Plasma concentration of IL-6 were significantly higher in SA than WT and BT ($P<0.001$). However no significant difference in IL-6 levels observed between WT and BT in all points of measurements.

In addition, IL-6 levels were significantly higher at P2 than P1 and P3 ($P<0.001$), but no significant difference was observed between P1 and P3.

Table 2: Plasma Interleukin-6 (IL-6) Levels Measured Before (P1), Immediately after (P2), and 60 Min after (P3) the Wingate Test in the South African, Black Tunisian, and White Tunisian at 08:00 and 18:00 H

	White Tunisian		Black Tunisian		South African	
	08:00 h	**18:00 h**	**08:00 h**	**18:00 h**	**08:00 h**	**18:00 h**
P1($pg.ml^{-1}$)	1.13±0.5	2.37±0.93	1.33±0.45	2.49±1.04	1.70±0.52	3.65±0.82 **¥**
P2($pg.ml^{-1}$)	5.65±1.48*****	7.11±1.40*	5.45±1.44*****	7.02±4.42*****	7.13±1.05*	8.35±0.89***¥**
P3($pg.ml^{-1}$)	1.83±0.65	4.11±0.99	2.5±0.68	3.35±1.02	1.79±0.4	4.22±0.35 **¥**

*: Significant different with P1

Significant different with morning; ¥

Significant different with Black Tunisian and White Tunisian

Anaerobic Performance

For P_{peak} and P_{mean} in the Wingate test, the ANOVA revealed significant main effects for groups ($F_{(2,14)} = 10.61$ and $F_{(2,14)} = 6.04$ respectively, $p<0.01$) and time-of-day ($F_{(1,7)} = 74.56$ and $F_{(1,7)} = 40.03$ respectively, $p<0.01$); however, no significant interaction of time-of-day × group ($F_{(2,14)} = 1.39$ and $F_{(2,14)} = 1.55$ respectively, $p>0.05$) was found. The post-hoc test revealed that P_{peak} and P_{mean} were significantly higher in the afternoon then the morning in all groups ($p<0.05$). Moreover, P_{peak} was significantly higher ($p<0.05$) in SA than WT and BT, with no significant difference between BT and WT (Figure 1).

For the FI, the main effects for time-of-day ($F_{(1.7)} = 1.67$, $p>0.05$) and groups ($F_{(2.14)} = 1.03$, $p>0.05$) and the interaction of time of day × groups($F_{(2.14)} = 0.42$, $p>0.05$) were not significant.

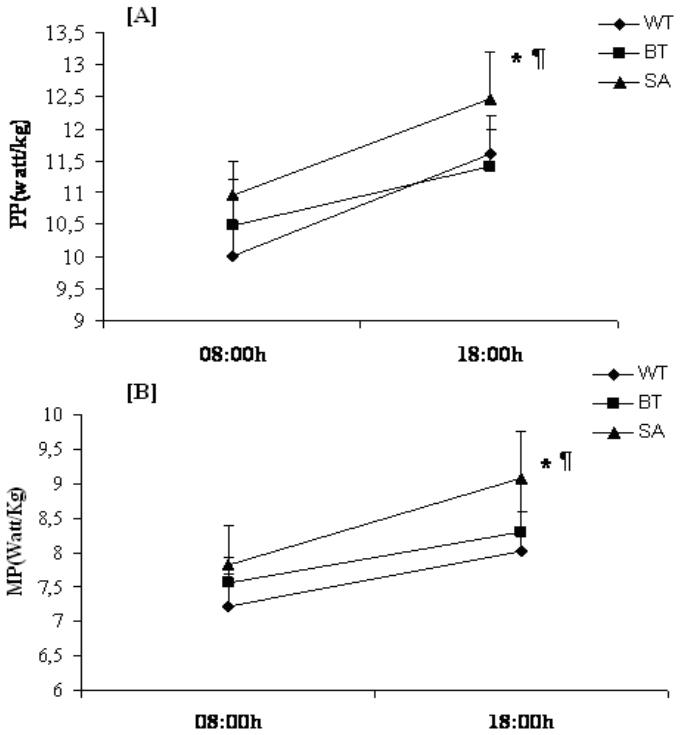

Figure 1: Peak Power (P_{Peak}) [A] and Mean Power (P_{Mean}) [B] Recorded at the Two Times-of-Day (I.E., 08:00 and 18:00 H) in the South African (SA), the Black Tunisian (BT), and the White Tunisian (WT) Soccer Players. Significant Different with Morning * Significant Different with BT and WT

Oral Temperature

A significant main effects of time of day ($F_{(2.14)} = 5.65$, $p<0.05$) and groups ($F_{(2.14)} = 19.7$, $p<0.001$) were observed, but no interaction effect between time of day × groups ($F_{(1.7)} = 0.29$, $p>0.05$) was found. The post-hoc test revealed that oral temperature was significantly higher in the afternoon and the evening in SA than WT and BT ($p<0.05$) (Figure 2).

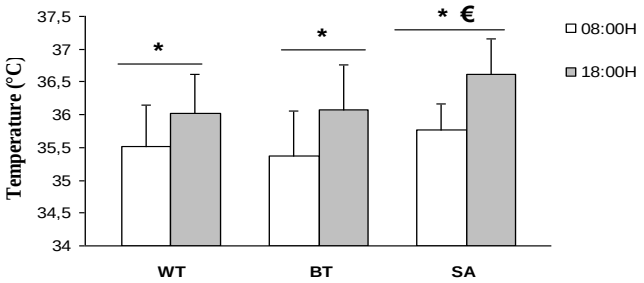

Figure 2: Oral Temperature Recorded at the Two Times-of-Day (I.E., 08:00 and 18:00 H) in the South African (SA), the Black Tunisian (BT), and the White Tunisian (WT) Soccer Players. * Significant Different with Morning. € Significant Different with BT and WT

DISCUSSIONS

The main purpose of this study was to explore the effects of time-of-day and race on anaerobic power and IL-6 levels in soccer players. The results showed that diurnal fluctuation of IL-6 was different between ethnic groups. Indeed, IL-6 level is higher during the afternoon hours in SA than WT and BT at P2. In addition, SA soccer players developed a higher anaerobic power during the 30-s Wingate test especially in the late afternoon (i.e., 18:00 h) which was around the peak of the circadian rhythm of body temperature.

The Effect of Race on Short-Term Maximal Performances

This finding indicates that the dominance of SA on anaerobic performance sports can be explained by qualitative factors such as motivation, socioeconomic and training status, and muscle fibre composition.

Consistent with **Ama et al., (1986)** the results of the present study confirmed the dominance of African athletes over the Tunisians (i.e., both WT and BT) in the short-term maximal exercises. Likewise, recently we found that P_{peak} and P_{mean} are higher in black than white subjects **(Abedelmalek et al., 2012b)**. This supremacy could be explained by motivation, socioeconomic and training status, and muscle fibre composition. Indeed, previous studies suggested that African had a higher percentage of fast-twitch fibres **(Ama at al., 1990)** and greater glycolytic enzyme activity responsible for the production of energy from the anaerobic metabolism **(Boulay et al., 1988)**. In addition, the results of present study indicated that the FI was not significantly different between SA, BT, and WT. However, previous results revealed that black subjects experience a greater degree of fatigue than whites during an anaerobic exercise **(Rahmani et al., 2004)**. **Rahmani et al., (2004)** explain this higher muscle fatigue in black compared to white subjects by the higher percentage of fast-twitch fibres. Indeed, fast-twitch fibres are more rapidly exhausted than slow-twitch fibres during high-speed contractions.

Moreover, the results of the present study demonstrated that performances of WT and BT during the Wingate test were not different. It has been shown that the geographical division between different groups of people, a partial consequence of racial and economic residential segregation, and environmental background are the main causes of the difference on physical performance **(De Jonge et al., 1996)**. Thus, it is not surprising to find similar anaerobic performances in WT and BT.

The Effects of Time-of-Day and Race on Short-Term Maximal Performances

Consistent with previous studies, **(Souissi et al., 2007a,b,2008)** the present study results showed that in all ethnic groups the P_{peak} and P_{mean} were significantly higher in the afternoon than the morning, which was around the peak of the circadian rhythm of body temperature. The diurnal increase in body temperature may exert a beneficial passive warm-up that may enhance metabolic reactions, increase the extensibility of connective tissue, reduce muscle viscosity, and increase conduction velocity of action potentials **(Souissi et al., 2002, 2003)**. In this context, **Chtourou et al., (2012a)** showed that the diurnal variations in maximal short-term performance during the Wingate test were linked to peripheral mechanisms, rather than to variation in central nervous command. Moreover, **Souissi et al., (2007a)** indicated that the time-of-day effect on performances during the Wingate test was partly due to better aerobic participation in energy production during the afternoon than the morning.

Furthermore, our results showed that SA demonstrated a higher P_{peak} and P_{mean} in the afternoon in comparison with BT and WT; however, no significant difference was observed between BT and WT. As this is the first study examining the effects of race and time-of-day on anaerobic power, it is difficult to compare our results with the current literature. However, we could speculate some possible explanations for such difference. Indeed, as afore mentioned, the time-of-day effect on performances during the Wingate test is partly due to better aerobic participation in energy production during the afternoon than the morning **(Souissi et al., 2007a)**. In this context, **Bosch et al., (1990)** showed that African males have a better ability to mobilize a higher percentage of maximal oxygen uptakes than whites. Another possible explanation, that the effect of time-of-day on muscle contractile properties could be attributed to the circadian rhythm in central temperature **(Souissi et al., 2004)**. Since oral temperature was significantly higher in the afternoon than the morning in SA than WT and BT, the higher value of P_{peak} and P_{mean} at 18:00 h in SA may be linked to

higher body temperature **(Chtourou et al., 2012b)**.

Concerning the muscle fatigue, our results show that the decrements in power (i.e., FI) were not affected by the time-of-day or by the racial variation. However, **Ama el al., (1990)** showed that blacks were less resistant to fatigue than whites and that power decrease appeared only after the 30-s of the anaerobic test (i.e., when large amounts of energy were derived from the muscle glycolysis) **(Di Prampero, 1981)**. Therefore, it is possible that the exercise duration in the present study was not long enough to cause a decrement in power.

Racial Variation of IL-6 during Short Term Maximal Exercise

Consistent with previous works **(Abedelmalek et al., 2012b; Meckel et al., 2009)**, the present results demonstrated that plasma IL-6 levels increase dramatically in response to exercise in all ethnic groups. Based on the common belief that the exercise-induced increase in IL-6 is a consequence of an immune response, it has been hypothesized that the immune cells are responsible for this increase **(Nehlsen - Canarella et al., 1997)**. Furthermore, our results showed that SA demonstrated higher plasma IL-6 concentration in comparison with BT and WT during short-term maximal exercise at P2. However, no significant difference of IL-6 concentrations was observed between BT and WT.

The results of present study were consistent with the study of **Chapman et al., (2009)** that showed considerable individual variation in inflammation between genders and between racial/ethnic groups. In this context, **Paalani et al., (2011)** showed that black subjects have higher IL-6 levels than white subjects at rest. However, the authors did not observe any significant differences between ethnics in IL-10 and TNF-alpha. In order to explain this supremacy, some authors have advanced the role of skeletal muscle histochemical and biochemical characteristics on the racial variations **(Ama et al., 1986; Green et al., 2006)**.

Plasma IL-6 and creatine kinase activity were elevated following intensified exercise. Moreover, **Terjung et al., (1985)** showed that the largest difference between racial groups was found at the level of creatine kinase activity and this fits well with a presumed advantage of Black males in the events of explosive and short duration.

In this context, previous studies indicated that the higher level of IL-6 was associated with muscle damage **(Miki et al., 1999)**; whereas muscle damage was followed by repair processes involving the migration of cytokine-releasing macrophages into the muscle. This explanation was further supported by **Halson et al., (2003)** who observed that the creatine kinase activity was higher in blacks than whites indicating possible mechanical injury to the musculoskeletal system that may induce an inflammatory response and subsequently an IL-6 response (Vgontzas et al., 2004).

Race/Ethnicity and Diurnal IL-6 Change during Exercise

Our results suggested that SA had higher levels of IL-6 at both times-of-day (i.e., especially in the evening hours) in comparison with WT and BT. These differences can be explained by numerous factors such as socioeconomic status. Because of evidence that socioeconomic status may play a bigger role in the psychological and physical health of blacks than whites, it is also possible that socioeconomic status interacts with race in predicting IL-6 in a similar manner.

In the same way, **Cohen et al., (2006)** showed that blacks had higher levels of cortisol during the evening and this association was similar for men and women. The authors found evidence of associations of daily cortisol profiles with race/ethnicity and socioeconomic status. In this context, **Späth-Schwalbe (1998)** found association with IL-6 that stimulated the adrenocorticotropic and cortisol hormones. Since physical activity may also be associated with pro-inflammatory changes in the autonomic nervous system and the hypothalamic-pituitary-adrenal axis, we could

speculate that higher levels of IL-6 in SA especially after the afternoon exercise could be related to the increases in the adrenocorticotropic and cortisol hormones.

Moreover, **Helge et al., (2003)** demonstrated that the release of IL-6 from working skeletal muscle was related to the exercise intensity. Thus, it is possible that larger increase in IL-6 levels on black subjects may occur during the afternoon exercise session due to the higher P_{peak} and P_{mean} developed by the SA as compared with BT and WT subjects.

Furthermore, **Barbosa et al., (2010)** explained the differences between races in muscle performances and biochemical parameters by the differences in molecular circadian clock. Indeed, the function of the biological clock was to supply temporal information to the organism so that physiological and/or behavioural responses can be coordinated during the daily cycle to maximize adaptability.

Based on previous studies **(Cruciani et al., 2008; Ciarleglio et al., 2008)** and the present findings and considering the history of human migrations around the planet over time, as well as the special characteristics of sunshine and light inherent to each place on earth, it is worth questioning which factors contributed to genetic variations that are specific to each ethnic group, especially considering the clock genes. Since the circadian phenotype is a result of strong interaction between the environment and the organism and the most important Zeitgeber for humans is the light/dark cycle given by the sun **(Roenneberg et al., 2009)**. It would be logical to find similar diurnal variation of IL-6 between WT and BT.

CONCLUSIONS

Our results showed that the diurnal fluctuation of IL-6 was different between ethnic groups. Indeed, immediately after 30-s anaerobic exercise the IL-6 levels were higher during the afternoon in SA than WT and BT. In addition, SA soccer players developed a higher muscle power during the 30-s anaerobic test especially when the short-term maximal exercises were performed in the late afternoon (i.e., 18:00 h) which was around the peak of the circadian rhythm of body temperature. The response of pro-inflammatory cytokines to exercise during the day can be learned by the athlete, coach, and his or her supporting stuff and may be used as an objective tool to monitor the training load and to better plan training cycles throughout the training season and competition period.

Study Limitations

The following suggestions would further improve this study: a) a larger participant pool; b) a broader range of age of participants; c) participants who compete in a wide range of sports; and d) the inclusion of female participants. The methodological approach being used in this study may have caused a limitation in regard to specific information provided by the participants. Because of the limited sample size, other important inflammatory (e.g., TNF-α) and hormonal (e.g., insulin-like growth factor-1) markers were not examined.

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